Title: Hepatocellular carcinoma cell lines growth inhibition by liver-derived mesenchymal stem cells in direct 3D co-culture

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Abstract (300 words):
Background and Aim: Hepatocellular Carcinoma (HCC) is the third highest cause of cancer-related death. Recently, cell therapy using mesenchymal stem cells (MSCs) is being explored as a potential new therapeutic strategy in oncology. MSCs display innate anti-tumoral properties *in vivo* and *in vitro* but their exact role in tumor emergence and progression is still debated, as other studies also highlighted pro-tumoral effects. Human Adult Liver Progenitor Cells (HALPCs), a liver-derived population of mesenchymal stem cells, are being developed as allogeneic products. The aim of this work is to investigate the therapeutic properties of HALPCs towards HCC through the study of their interactions with three human HCC cell lines (HepG2, Hep3B and HuH7) in a 3D co-culture model.
Method: Spheroids were formed in ultra-low attachment plates. In co-culture spheroids, HALPCs and either HepG2, Hep3B or HuH7 cells were seeded at different cell-to-cell ratios to explore spheroid growth by daily brightfield micrography. Spheroid area measurement was automated using an ImageJ Macro. Cell proliferation was also assessed through luminometry-based measurement of spheroids' ATP content and Ki67 immunostaining on paraffin-embedded spheroid slices.
Results: The spheroids' size follow-up revealed a statistically significant decrease in spheroid growth velocity when HALPCs were co-cultured with the different cell lines. This effect was dependent on the HALPC ratio in the spheroid. Biochemical and histological analyses supported those results, with a significant decrease of the total ATP content and the number of Ki67 + cells in bi-cellular spheroids when compared to their respective controls.
Conclusion: In a 3D *in vitro* direct co-culture model, HALPCs are able to reduce the proliferation of three different human HCC cell lines (HepG2, Hep3B and HuH7). This effect was proven morphologically, biochemically and histologically. The deciphering of the mechanisms of action of these effects is ongoing while *in vivo* validation of these results is mandatory.

Figure:

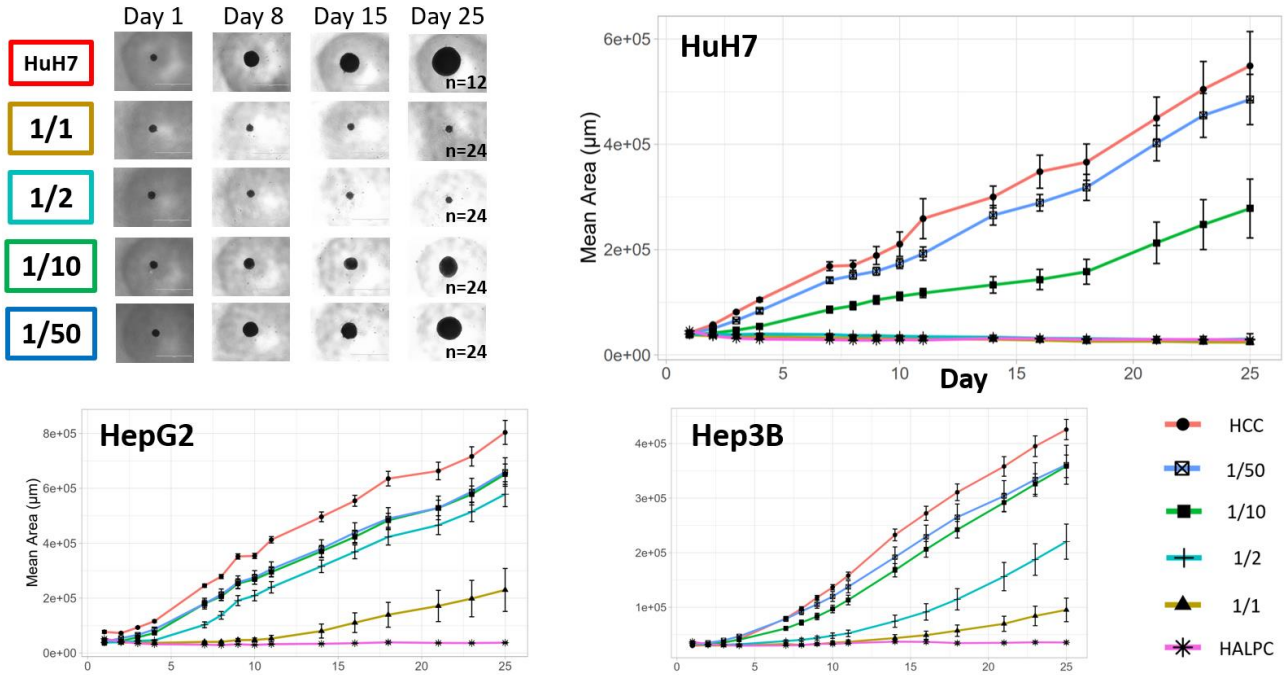

Figure 1: HepG2/HuH7/Hep3B and HALPC coculture spheroids growth follow-up

Method: Coculture spheroids were formed in u-bottom ultra-low attachment plates by seeding a total of 1000 cells/well at varying ratios of both cells types (*i.e*. 1/1 consists of 500 HALPC cells and 500 HepG2/HuH7/Hep3B cells, 1/2 consists of 333 HALPC cells and 666 HepG2/HuH7/Hep3B cells, 1/10 consists of 100 HALPC cells and 900 HepG2/HuH7/Hep3B cells and 1/50 consists of 20 HALPC cells and 980 HepG2/HuH7/Hep3B cells). Mono-cellular spheroids containing only one of the cell types were used as controls. Representative micrographs of each type of spheroid taken at different days after their formation are displayed to visually illustrate the difference in spheroid growth. Graphs show the mean area of each subtype of spheroid, measured using an automated process with an ImageJ macro each day after their formation, and their individual confidence interval.

Results: A statistically significant decrease in the size and the growth of the spheroids can be seen with each of the three cancer cell line when in the presence of HALPC. In HuH7, a complete stop of spheroid growth and even a decrease of the initial spheroid size can be seen at the 1/1 and 1/2 ratio. The intensity of the spheroid growth inhibition gradually fades in lower ratios, indicating the presence of a dose-response relationship.